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PEPTIDE SYNTHESIS BY PRIOR THIOL CAPTURE...III. ASSESSMENT OF LEVELS OF RACEMIZATION DURING TWO TYPICAL THIOL CAPTURE COUPLING REACTIONS

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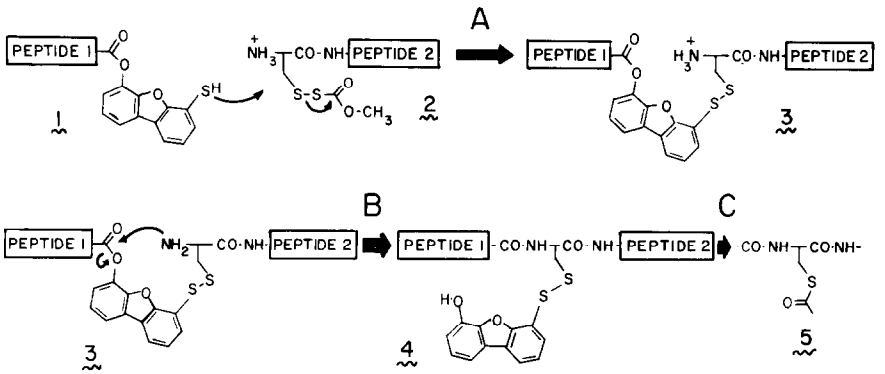
Abstract: Racemization during peptide bond formation by the dibenzofuran-based thiol capture strategy has been assessed through synthesis of two model peptides, Z-L-Ala-L-Ile-Cys-OMe and Z-L-Ala-L-Phe-L-Cys-OMaq. The former case gave (0.20 ± 0.27)% of the D-aIle epimer and the latter, less than 0.1% of the L-D-L-epimer.

Previously we have described design plans and preliminary results for an unconventional approach to peptide synthesis by prior thiol capture, summarized by the capture Step A, the acyl transfer Step B, and the S-S cleavage and S-blocking Steps C seen in Scheme I.[1] In effect, the thiol capture strategy provides a chemical version of a ligase for N-cysteine-bearing peptides, and it is expected to be most useful in linking medium-sized polypeptides that have been prepared by solid phase synthesis. In other papers in this series we consider the synthesis of peptides bearing C-terminal 4-dibenzofuran-6-thiol linkages,[2] the design of templates such as the 4,6-functionalized dibenzofurans that permit facile intramolecular O,N-acyl transfer (Step B),[3] formation of unsymmetrical disulfides 3 (Step A), control of disulfide interchange, and practical synthesis of large peptides by thiol capture. Here we consider the key problem of racemization.

Levels of racemization at a given amino acid site are usually insignificant (ca 0.03%) when peptides are synthesized by a linear strategy involving active acyl derivatives of N-urethane-blocked amino acids.[4] However, convergent syntheses, which must involve acylating agents that are derived from polypeptides, frequently encounter troublesome coupling steps in which racemization occurs an unacceptably high levels.[5] Since one of the attractive features of the thiol capture strategy is a probable freedom from racemization, it was important to provide experimental evidence in support of that expectation.

The diasteromeric amino acids L-isoleucine and D-allo-isoleucine can be conveniently separated on the ion exchange column of a conventional amino acid analyzer, and widely used

SCHEME I



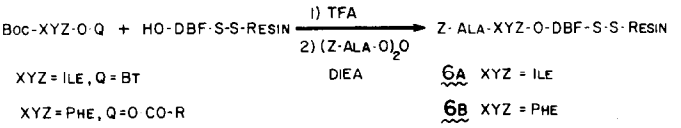
tests for racemization involve a three-step sequence -- coupling of an N-acyl-L-isoleucine to an amino acid ester, acidic hydrolysis to form the free amino acids, and assay of the Ile to aIle ratio.[6] Such tests are limited by the detection sensitivity of the analysis and by the need to correct for the significant epimerization that occurs during the high temperature conditions of the hydrolysis step.

As seen in Scheme II, the 4-acyloxydibenzofuran-6-thiol 1 (RCO_2-DBF-SH) derived from Z-Ala-Ile-OH was prepared by acylation of HO-DBF-S-S-Resin[7] by Z-Ile-OBt[7] in the presence of the hindered base diisopropylethylamine (DIEA), N-Boc cleavage with TFA, followed by a second acylation with $(Z\text{-}Ala)_2O$ with DIEA.[8] Disulfide cleavage with triethylphosphine (9:1 dioxane-water, 1 min.), evaporation, and reaction with BOC-Cys(Scm)-OMe[9] (1 equiv.) in wet hexafluoroisopropyl alcohol (HFIP) gave 7A after evaporation and flash chromatography. TFA treatment and precipitation (CH_2CL_2 - pet. ether) gave the corresponding amine salt as a powder,[10] 50% from HO-DBF-S-S-Resin. This salt was dissolved at 25°C in DMSO containing 2.5 equiv. DIEA and 0.2 equiv. Ph-Hg-OAc (to inhibit S-S interchange). After 3d, the solvent was removed, and 8A 60% was isolated by prep. TLC. Determination of the ratio of aIle to Ile in this sample was carried out after hydrolysis in 12 M HCl-propionic acid at 150°C[10] for a series of reaction times in the range of 5 to 30 min. Extent of epimerization of the Ile residue during the reaction sequence of Scheme II was obtained as the zero intercept of a plot of % aIle vs time of hydrolysis. A value of (0.20 ± 0.27)% was obtained by a linear least squares analysis. Thus, within the limits of accuracy of the method, no epimerization was detectable.

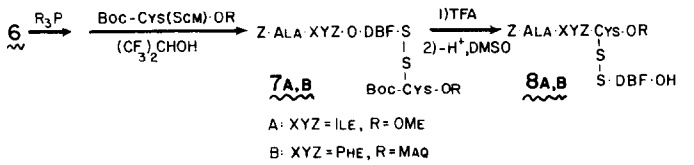
A more sensitive assay was available to us through the discovery that Z-L-Ala-L-Phe-L-Cys(S-DBF-OH)-OMaq (where Maq is the 2-oxymethylanthraquinone group[12]) can be separated from its LDL epimer with nearly baseline resolution by HPLC. The reaction sequence described above and reported in Scheme II was repeated replacing Ile by Phe and making the following changes: 1) $(Boc\text{-}Phe)_2O$ + DIEA was used in the first acylation, 2) Boc-Cys(Scm)-OMaq was used in the capture step, 3) the acyl transfer was complete in less than 5h, 4) the yield of 8B was 90%.[10]

SCHEME II

A. SYNTHESIS OF Z-Ala-Xyz-O-DBF-SH (DBF = 4,6-dibenzofuranyl)



B. CAPTURE AND ACYL TRANSFER



The resulting sample was compared by HPLC analysis with a series of standard mixtures of the pure LLL and LDL epimers. At a ratio of 99.95 to 0.05, the LDL epimer appeared as a barely detectible unresolved shoulder on the major peak, but was resolved as a separate peak at a ratio of 99.9 to 0.1 and at all higher ratios. Comparison of 8B with the standards showed its diastereomeric content to be less than 0.1% and comparable to that of the 0.05% standard which lies at the limit of detectability by this method.

It is noteworthy that in both Ile and Phe cases, the observed levels of epimers result from a series of racemizations encountered during the entire reaction sequence of Scheme II, which includes two acylation steps -- formation of the phenolic ester linkage to the template and the amide-forming intramolecular acyl transfer, both of which were deliberately carried out in the presence of an excess of the tertiary amine base DIEA. [13]

Racemization of peptide-derived acylating agents almost invariably occurs by a three-step mechanism [5] involving 1) intramolecular neighboring group participation of an amide function to form an azlactone, 2) epimerization of the azlactone, 3) peptide bond formation by the epimerized azlactone. Becuase the thiol capture strategy involves an intramolecular peptide bond formation step that is driven entropically by a high effective molarity of the cysteine amine function at the active ester carbonyl, racemization by an azlactone mechanism is expected to be inefficient for two reasons. First, the phenolic ester 3 is deliberately chosen to be a weak acylating agent, for which neighboring group participation by a weakly nucleophilic amide function is expected to be very sluggish. Second, since all azlactone must be formed by a fragmentation step, it must then compete intermolecularly in dilute solution with the intramolecular acyl transfer step. Gratifyingly, these design features of the thiol capture strategy have been confirmed experimentally by our failure to detect racemization at levels higher than the error limits of the two model systems.

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REFERENCES

1. Kemp, D.S., Biopolymers, (1981), 20, 1793; Kemp, D.S. Galakatos, N.; Bolin, D., in "Peptides: Synthesis, Structure, Function," D. Rich, E. Gross, eds., Pierce Chemical Co., Rockford, Ill., 1981, pp 73-75.
2. Kemp, D.S.; Galakatos, N.G.; J. Org. Chem., (1986), 51, 1821.
3. Kemp, D.S.; Galakatos, N.G.; Bowen, B.; Tan, K.; J. Org. Chem., (1986), 51, 1829.
4. Rebek, J.; Feitler, D., Int. J. Pept. Protein Res. (1975), 7, 167-169
5. Kemp, D.S. in "The Peptides," E. Gross, J. Meienhofer, eds., Academic Press, N.Y., 1979, vol. 1, p 342-378; Benoiton, N.L., in "The Peptides," E. Gross, J. Meienhofer, eds., Academic Press, N.Y., vol 5B, 1983, pp 218-279.
6. Bodanszky, M.; Conklin, L. (1967), Chem. Comm. 591-593. Kitada, C.; Fujino, M., Chem. Pharm. Bull. (1978), 26, 585.
7. As reported in ref 2, resin was prepared by reaction of 1% cross-linked chloromethylated polystyrene with ZCys(Trt)OCs, followed by Cl-S-CO-OMe (Scm-Cl), then by HO-DBF-SH. Preformed 1-hydroxybenzotriazole ester of Z-Ile-OH (DCC + acid + HOBt, CH_2Cl_2,-2°C) was combined with HO-DBF-S-S-Resin and DIEA (equiv: 4:1:4) at 0°C for 35 min, washed[2], treated with 1:1 TFA-CH_2CL_2 (30 min.), and acylated with preformed[8] $(Z\text{-}Ala)_2O$ in the presence of DIEA.
8. Meienhofer, J.; Waki, M.; Heimer,E.;. Lambros, T.; Makofske, R.; Chang, C-D., Int. J. Pept. Protein Res., (1979), 13, 35.
9. Hiskey, R.G.; Muthukumaramaswamy, N.; Vunnam, R. J. Org. Chem. (1975), 40, 950.
10. 3 from 7A was homogeneous by TLC and HPLC; char.: A.A. analysis: Ala, 0.97; Ile, 1.0. (Cys not det.). FAB MS: Calc for M^+: 668; Found: 668. 8A was char. by AA analysis, ^1H NMR, FAB MS: Found for M^+: 668, with some symm. disulfide of Z-Ala-Ile-Cys-OMe (905). 3 from 7B m.p. 164-167°C.; A.A. anal; Ala, 1.0; Phe, 1.0; char. by ^1H NMR, yield 57% from HO-DBF-S-S-Resin. 8B char by A.A. anal: Ala, 1.0; Phe, 0.97; ^1H NMR; FAB MS: Calc. for M^+ + DIEA: 1036; Found: 1036. Calc for M^+, 907; Found: 907.
11. Scotchler, J.; Lozier, R.; Robinson, A., J. Org. Chem., (1970), 35, 3151-3152
12. Kemp, D.; Reczek, J. Tetrahedron Lett., (1977), 12, 1031-1034.
13. The inefficiency of DIEA for simple epimerization was noted by Bodanszky: Bodanszky, M.; Bodanszky, A. Chem. Comm., (1967), 591-593. See also, ref. 5B., pp 243 for discussion.

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